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A NONSPATIAL CONCURRENT DISCRIMINATION LEARNING TEST FOR A RAT MODEL OF THE NEUROPSYCHOLOGY OF MEMORY

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The concurrent discrimination learning paradigm to train animals on a number of two-choice discrimination problems in a concurrent and parallel way has long been shown a sensitive test of memory alteration in a monkey model for the neuropsychology of memory. An attempt was made in the present study to train rats on a nonspatial concurrent discrimination learning of 6 or 8 problems (pairs) in a Y-maze to develop an analogue of the concurrent learning paradigm for a rat model. Sixteen stimulus boxes varied both visually and tactually were used as nonspatial discriminative stimuli, forming 6 or 8 pairs. Each pair was presented equally often in a daily session, the placement of the positive stimulus being randomized between the right and left positions, until the overall criterion was achieved. The concurrent learning of 8 pairs as well as that of 6 pairs was completed by all of the assigned rats. The present concurrent learning was easier to learn compared with those recently reported by two other rat studies.

Key words: concurrent discrimination learning, memory test, rat

Rats and monkeys are two most popular animal models for the neuropsychology of memory. The selection of appropriate behavioral testings is a critical factor affecting outcomes of neurobiological experiments. In a monkey model, two types of memory tests have been established to be useful tests sensitive to the medial temporal lobe amnesia which is thought largely due to dysfunction in the hippocampal system. One type of test (Mishkin, 1978) is a delayed nonmatching-to-sample (DNMS) task in which monkeys are required to choose a new comparison object against a sample object presented to them a short time earlier, thus to utilize recognition memory for a correct responding. The other type (Moss, Mahut, & Zola-Morgan, 1981) is called a concurrent discrimination learning, where animals have to learn a number of two-choice object discriminations in a concurrent and parallel way. That is, all the discrimination problems are presented equal times in a daily session, and this procedure is repeated until the animals attain a criterion that requires them to perform at a high level of accuracy on each problem at the same time. Thus, the concurrent learning is sharply contrasted with an easier, serial discrimination learning of the same number of discrimination problems, in which animals are trained on one problem at one time and moved to the next problem after they have completed the preceding one (Kikuchi & Iwai, 1980). It is important to note that the memory performance examined in these two tests well resembls human mnemonic activities in everyday life. Following a dichotomy of working memory and

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reference memory (Olton, Becker, & Handelmann, 1980), the first type of test demands mainly working memory, and the second type eference memory.

Many rat experiments have shown that the first type of test and its variations are also effective to detect memory impairment resulting from the hippocampal dysfunction in the rat (for example, Aggleton, Hunt, & Rawlins, 1986; Aggleton, Blindt, & Rawlins, 1989; Kesner, Bolland, & Dakis, 1993; Olton, Walker, & Wolf, 1982; Peinado-Manzano, 1990; Rasmussen, Barnes, & McNaughton, 1989; Rothblat & Kromer, 1991; Thomas & Gash, 1986). However, these positive findings, obtained reliably from spatial memory tasks, are seriously questioned by the facts that neurons in the rat hippocampus are found to code spatial locations (Muller, Kubie, & Rank, 1987; O'Keefe & Dostrovsky, 1971; Olton, Branch, & Best, 1978) and that lesions in the rat hippocampal system produce substantial impairment on any memory and learning tasks involving spatial information processing, ranging from a single two-choice discrimination learning (Becker et al., 1981; Schenk & Morris, 1985) to complex tasks such as spatial DNMS (Aggleton et al., 1986, 1989; Peinado-Manzano, 1990; Rothblat & Kromer, 1991; Thomas & Gash, 1986) and radial maze tests (Olton et al., 1982; Rasmussen et al., 1989). Behavioral impairment following hippocampal lesions in rats, therefore, could be rightly interpreted to reflect spatial cognitive dysfunction but not memory dysfunction (O'Keefe et al., 1975). To dissociate memory dysfunction from cognitive dysfunction, it is needed to use nonspatial versions of DNMS and concurrent learning paradigms which demand no spatial cognition for a critical component (Olton et al., 1980). Responding to this need, there have been invented various analogues of nonspatial DNMS or DNM(delayed matching-to-sample) tests for a rat model. Results obtained from ablation studies using newly developed nonspatial memory tests are equivocal, some ones (Aggleton et al., 1989; Raffaele & Olton, 1988; Wood et al., 1993) indicating memory impairment but many others (Aggleton et al., 1986; Kesner et al., 1993; Mumby, Wood, & Pinel, 1992; Peinado-Manzano, 1990; Rasmussen et al., 1989; Rothblat & Kromer, 1991; Sutherland & McDonald, 1990) essentially no impairment after lesions in the hippocampal system.

The development of an analogue of the concurrent discrimination learning, the second sensitive memory test in monkey models, has just started with only two reports published (Aggleton, Kentridge, & Sembi, 1991; Wible, Shiber, & Olton, 1992). The present study reported a nonspatial concurrent discrimination learning for a rat model which is relatively easy to learn and advantageous in controlling possible involvement of rodent subjects' odor trail into test performance in a maze task.

METHODS

Subjects

The subjects were 11 experimentally naive, male Wistar rat 4 months old at the start of the experiment. They were housed individually in standard rodent cages in a temperature-controlled room. Water was always available ad lib, but food intake was restricted during the experiment. The body weight was reduced to about 85% of the freely feeding weight and was kept at this level throughout the experiment. The training was conducted in the afternoon

in a laboratory room where the ambient temperature was maintained at 23℃ ±2°. The rats were divided into two groups, one group(N = 5) allocated to a 6-pair concurrent discrimination learning and the other(N = 6) to a 8-pair concurrent discrimination learning, matched for learning scores on a visual pattern discrimination learning.

Apparatus

A trough wooden Y-maze was used which consisted of a start stem, two goal arms, and a choice area(junction), each painted gray (Fig. 1). A guillotine door was located at the exit of the start stem (24cm long × 13cm wide × 24cm high), and the entrance to each goal arm (41cm long × 13cm wide × 24cm high). The top of the choice area (33cm long; two width dimensions of 13 and 28cm; 24cm high) was closed with an acrylic mesh sheet, and that of the start stem was covered by a hinged lid made of acrylic mesh. Each goal arm had a clear Plexiglas hinged lid on the top. The far end of each goal arm was open so as to allow inserting a stimulus box (discriminanda) into the inside and had a bridge 14cm above the floor. On the bridge was situated a food well (25mm in inner diameter and 8mm deep) which could be covered by an opaque lid light enough to be easily displaced by a rat. The stimulus boxes (39cm long × 12cm wide × 13cm high) were constructed of sheet aluminum and had the top and one end opened. When they were inserted onto a goal arm, their open ends bordered just on the choice area.

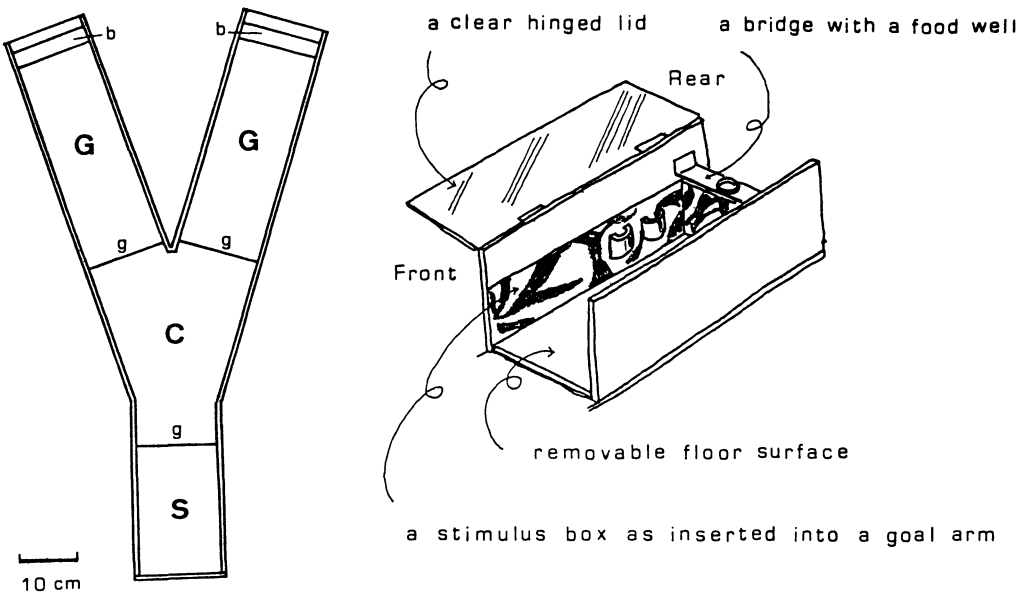


Fig. 1. A scale diagram of the Y-maze (on the left) and an illustration to show how a stimulus box was placed into a goal arm (on the right). Abbreviations for the scale diagram: C, choice area; G, goal arm; S, start stem; b, bridge; g, guillotine door.

Three types of stimulus boxes were prepared. The first type included only a pair of identical, black-painted stimulus boxes to be used in the maze adaptation. The second type also contained only a pair of stimulus boxes, which differed in pattern painted on the inner

walls (white stripes vs. white circles) and served in a visual pattern discrimination learning. Sixteen stimulus boxes belonging to the final type (Fig. 1) were varied in the color, pattern, texture of the surface sheets pasted on the inner walls, and were made further distinctive by adding small junk objects (e.g., spoon, cup, bracket and wooden block) attached to the walls.

Forming 6 or 8 pairs, they served as discriminanda in the concurrent discrimination learning. Two identical removable plates made of gray vinyl plastic were employed to form the floor surface of each stimulus box from any pair, their right-left positions being unvaried across trials and subjects. The aim of this procedure was to minimize possible involvement of rodent subject's odor trail into test performance on the present maze task (see Discussion).

The illumination was from two 40-W fluorescent bulbs at the ceiling of the room through a translucent paper sheet, providing a diffuse light of 240 lux at the level of the maze apparatus which was elevated 51 cm from the floor of the room.

Behavioral Procedure

Following handling, the rats were given the maze adaptation training using a pair of identical black-painted stimulus boxes. Each animal was shaped to run down from the start stem to the far end of the goal arm and to obtain food pellets from the food well on the bridge by displacing the overlying lid. They received adaptation trials equally often in each goal arm.After the completion of the maze adaptation, all rats were trained on a visual pattern discrimination learning where they were required to discriminate between two white-and-black patterns and attain a criterion of 100% correct in a single block of 10 trials.

The concurrent discrimination learning followed the pattern discrimination learning which was completed with a mean of 164 trials. The rats were divided into two groups matched for learning scores on the preceding pattern learning, and one group (N = 5) was trained on the concurrent learning of 6 two-choice discriminations (6-pair concurrent discrimination learning) and the other group (N = 6) on that of 8 two-choice discriminations (8-pair concurrent discrimination learning). The discrimination problems used were 8 fixed pairs formed from 16 stimulus boxes distinctive in color, pattern, texture, and small junk objects they contained on the walls. All of them were used for the 8-pair concurrent learning, and 6 out of them for the 6-pair concurrent learning. Preferred stimulus boxes were designated as the positive stimuli in half of the pairs, and nonpreferred ones in the remaining half, in each concurrent discrimination learning. The preferred positive stimulus was the member of a pair a rat chose on the first-confronted trial for that pair, in which both goal arms were exceptionally baited. The nonpreferred positive stimulus was the member of a pair the rat did not choose on the first-presented trial for that pair, where neither goal arm was baited. A daily session consisted of 4 blocks of 6 trials each (one trial per pair) amounting to total 24 trials in the 6-pair concurrent learning, and of 3 blocks of 8 trials each (one trial per pair) amounting to the same number of 24 trials in the 8-pair concurrent learning. The intertrial interval was about 1 min. in both learnings. The pairs were presented in a given order in every block for 3 subjects and in the reversed order for the remaining subjects in each training group. A correct response, the choice of the goal arm containing the positive stimulus box,

was rewarded with two food pellets (72mg in total weight) in the food well. A wrong response, the choice of the goal arm containing the negative stimulus or the failure in making a choice within 3 min., was followed by no reward. After making a wrong choice, the rat was confined in the goal arm for 10 sec before being returned to the holding cage. No correction trials were allowed. A choice response was defined as the animal's entering either goal arm with four paws. The guillotine door behind the animal was closed by pulling strings to prevent its retracing. The placement of the positive stimulus box on the right and left goal arms was determined pseudorandomly with the restrictions that the positive stimuli appeared equal times in each goal arm over total 24 trials of a daily session and that 4 or more trials of the same placement in succession were avoided. Concerning a particular pair as well, the placement of the positive stimulus was equalized for each goal arm over two daily sessions. The training was continued until the rat achieved a criterion of 47 correct responses over two consecutive daily sessions (i.e., one error in 48 trials), in either concurrent learning.

Results

The 6-pair concurrent discrimination learning

All of the rats completed the concurrent discrimination learning within 16 daily sessions with mean scores of 259 trials and 81 errors to criterion (Fig. 2). The most rapid learner required 216 trials and 71 errors, and the slowest did 336 trials and 101 errors (Fig. 3A). The difference in individual learning curves became conspicuous mainly after the period of initial improvement up to a relatively high performance level (80% correct in a daily session):

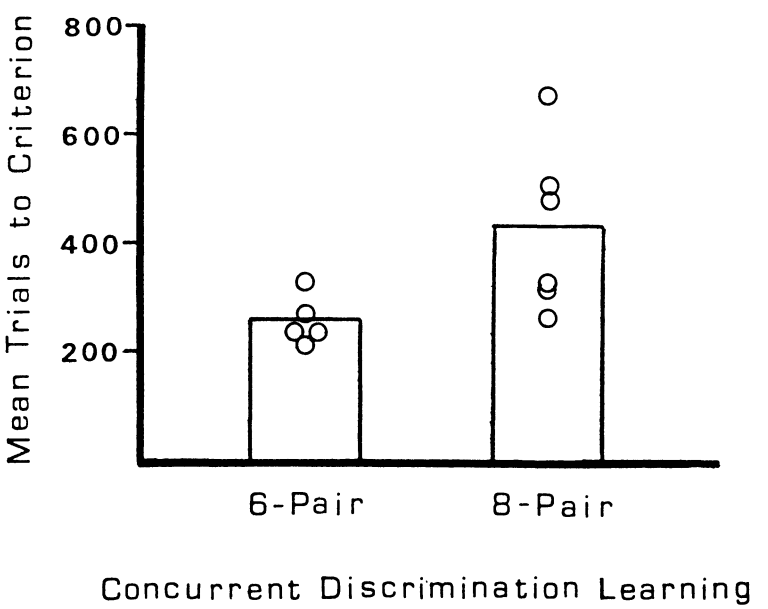
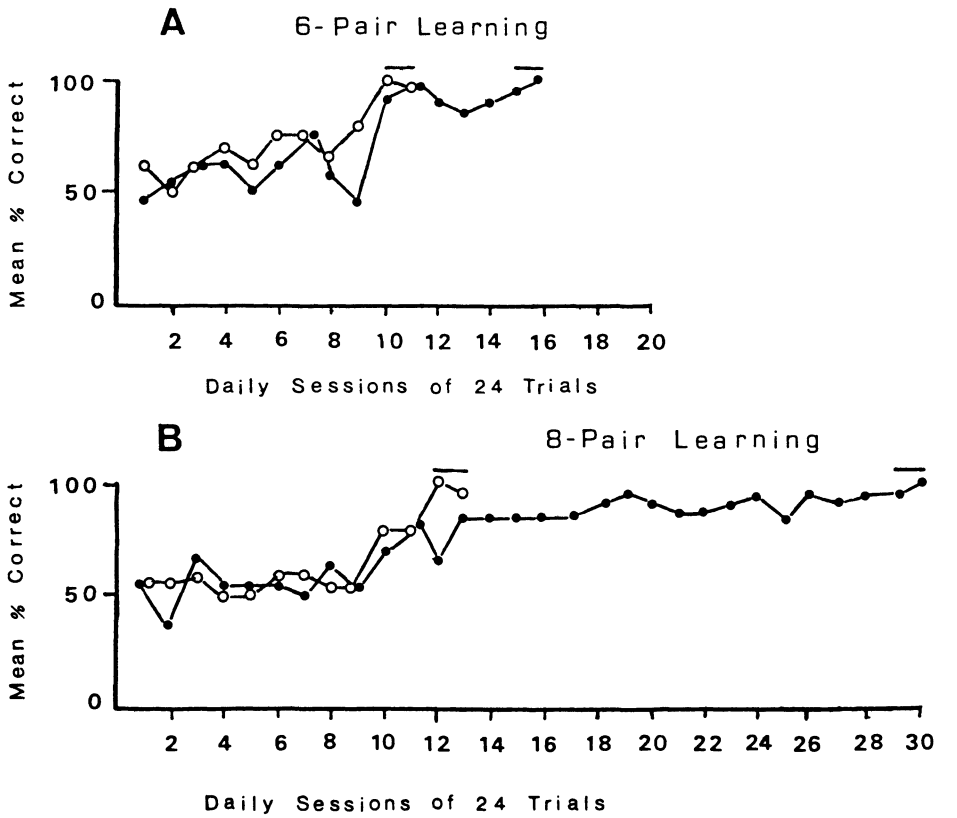


Fig. 2. Mean learning scores on the nonspatial concurrent discrimination learnings of 6 pairs and 8 pairs by rats. Open circles show learning scores for individual rats.



Figs. 3A. and 3B. The learning curves shown by the most rapid (open circle) and the slowest learner (filled circle) on each of the nonspatial concurrent discrimination learnings of 6 pairs (A) and 8 pairs (B). Bars above learning curves indicate the criterion performance. Note that the most rapid and the slowest rat, on each learning task, largely diverged after having reached a high level of 80 % correct performance at a similar rate.

after showing the initial improvement at a similar rate, as typically depicted in Fig. 3A, slower rats took longer to reach the criterion level than faster rats.

The 8-pair concurrent discrimination learning

This concurrent learning also was successfully learnt by all the rats, with mean scores of 428 trials and 129 errors, within 30 daily sessions (Fig. 2). The most rapid learner required 264 trials and 108 errors, and the slowest did 672 trials and 156 errors (Fig. 3B). As exemplified in Fig. 3B, the same pattern of difference in learning curves following the initial improvement was observed, but this time more clearly than when in the 6-pair learning. It was unlikely that a prolonged time needed by slower learners to elevate a high level of 80 % correct performance up to the final criterion level might be owing to a single problem (pair) which was very difficult to solve. For, when the learning data for each discrimination problem was separately analyzed with a criterion of 7 corrects over 2 consecutive training days (7 corrects in 8 trials for individual problems of the 6-pair concurrent learning) or 8 corrects over

3 consecutive days (8 corrects in 9 trials for those of the 8-pair concurrent learning), the most difficult problem (i.e., the last-learnt problem) was mastered long before the concurrent criterion was attained. The slowest rat on the 8-pair learning, for example, had mastered the most difficult pair on the 22 th training day, about one week before it achieved the concurrent criterion (Fig. 3B).

A simple comparison between the two concurrent learnings by a one-tailed Mann-Whitney U test indicated that the 8-pair learning took more numerous trials and errors than the 6-pair learning (for trials, $U = 3$, $p = 0.015$; for errors, $U = 2$, $p = 0.009$). For the purpose of camparing in other way, the learning scores were divided by the number of discrimination problems (pairs) to produce learning scores per problem (pair). New learning scores were largely overlapped between the groups, thus failing to show any significant group difference in trial or error scores. It is, however, worth noting that the worst three of new learning scores across groups were all obtained from animals of the 8-pair learning group for each of trial and error scores.

Discussion

Responding to the need of versions for a rat model of the amnesia-sensitive memory tests established in monkey experiments, an attempt was made to train rats on a nonspatial concurrent discrimination learning of 6 or 8 problems (pairs) in a Y-maze. For the discriminative stimuli were used stimulus boxes whose inner walls differed in color, pattern, texture, and small objects fastened, thus providing salient visual and tactile cues, and less salient olfactory cue probably originating from the surface sheet and objects. The concurrent learning of 6 problems (6-pair concurrent learning) was learnt without difficulty, and even the concurrent learning of 8 problems (8-pair concurrent learning) was completed by all the rats within 30 training days. The present results were well contrasted with those recently reported by two other studies which similarly trained rats on a nonspatial concurrent discrimination learning (Aggleton et al., 1991; Wible et al., 1992).

Aggleton et al. (1991) trained rats (AD strain) on a 6-pair concurrent discrimination learning on a modified Y-maze, using objects made of painted wood and sealed with coats of clear lacquer paints as the discriminative stimuli. The rats could find reward pellets in the food well by pushing aside the overlying positive object when they chose the correct side of the goal area which containing that positive object. The training began after the completion of a complex object discrimination learning and continued for 36 days with a daily session of 24 trials (4 trials per pair). Eighteen rats, as a group, reached a level of over 90% correct performance in a daily session on the very last training day (i.e., on the 36 th daily session), requiring a much longer time compared with the rats of the 6-pair learning group in the present study who, as a group, achieved the same performance level on the 11 th training day (Fig. 3A). If disregarding some differences in rat strain, discriminanda, and apparatus, it may be said that the present nonspatial concurrent learning is easier to acquire.

Wible et al. (1992) trained Long-Evans rats on a concurrent discrimination learning of 8 pairs of junk objects in a similarly modified Y-maze, for a total of 60 daily sessions, following

the completion of two standard two-choice object discrimination learnings. The animals had to go behind the positive object placed at one side of the goal area in order to get the reward.Five out of the 7 trained rats attained a criterion of 100 % correct responding over a daily session of 40 trials (5 trials per pair), nearly comparable to the criterion of 47 correct responses in 48 trials for the 8-pair concurrent learning in the present study, with a calculated mean score of 340 errors from Fig. 7. in that paper, about 2.5 times as large as the mean score of 129 errors for the present 8-pair concurrent learning group. A reanalysis of the learning data with a criterion of 39 correct responses in 40 trials indicated that one out of the 7 rats still failed to reach the new criterion. Despite some procedural differences between the two studies, it may be said again that the present concurrent discrimination learning test is easier for a rat to learn than the one used by Wible et al. Moreover, it is of some interest to note that the rats in the present study completed the 8-pair concurrent learning at about the same rate as macaque monkeys did a concurrent discrimination learning of the same number of pairs of junk objects (Zola-Morgan et al., 1993). That is, monkeys in that study were given 40 trials (5 trials per pair) for a daily session until attaining a criterion of 39 correct responses in 40 trials, a criterion comparable to that for the present rats, and they obtained a mean score of 468 trials to criterion not different from that of 428 trials obtained by the present rats.

To control possible contamination by rodent subjects' odor trail into test performance is a painstaking work commonly to be performed in a maze training (Olton & Samuelson, 1976; Thomas & Gash, 1986). The present study used stimulus boxes with two identical removable floor surfaces in order to eliminate inadvertent cues for discrimination performance possibly provided by the odor trail a rat subject might otherwise have left on the floors of stimulus boxes. The invariant allocation of these removable floor surfaces to the right and left positions prevented their placement from being associated with the placement of the positive and negative stimulus boxes varied between the right and left goal arms from trial to trial, so that a most important source of the odor trail cuing could be excluded. This effective but less painstaking procedure is another advantage in the present version of the concurrent discrimination learning.

What remains to be done is to examine how competently the present version could reflect memory alteration following damage to the rat brain. Both of the versions by Aggleton et al.and by Wible et al. were shown sensitive to memory disorders produced by lesions in the rat hippocampal system (Aggleton et al., 1991; Wible et al., 1992).

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